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NEURONAL CONTROL OF CILIARY LOCOMOTION IN A GASTROPOD VELIGER (*CALLIOSTOMA*)

S. A. ARKETT, G. O. MACKIE, AND C. L. SINGLA

Department of Biology, University of Victoria, Victoria, British Columbia V8W 2Y2

ABSTRACT

Intracellular recordings from pre-oral ciliated cells of competent *Calliostoma ligatum* veligers were used to demonstrate the mechanisms of neuronal control of ciliary locomotion. During normal ciliary beating at 5–7 Hz, the membrane potential shows no oscillations or spiking activity. It remains at a resting potential of about −60 mV. Depolarization from resting potential is due to excitatory input from the CNS and, depending upon the kind of input, veligers appear to show two types of locomotory behavior. In one type, normal ciliary beating is periodically interrupted by rapid, velum-wide ciliary arrests. These arrests are caused by a propagated, Ca^{++}-dependent action potential in the pre-oral ciliated cells. The second type is characterized by either a velum-wide or local slowing of normal ciliary beating, and appears to result from a slow depolarization of the ciliated cell membrane. Pre-oral ciliated cells are electrically coupled to each other. This property may ensure the synchrony of velum-wide ciliary arrests or differential velar slowing of ciliary beating. These findings demonstrate some of the mechanisms of the fine control veligers possess over their locomotory and feeding behavior.

INTRODUCTION

Many metazoans that use cilia to move or to produce feeding currents can control the frequency of ciliary beating. This control is often revealed as intermittent, rapid arrests of ciliary beating. Ciliary arrests have been correlated with chemically mediated, rapid, postsynaptic depolarizations of the ciliated cell membrane (Mackie *et al.*, 1974; Murakami and Takahashi, 1975; Mackie *et al.*, 1976; Moss and Tamm, 1986; Arkett, 1987). These depolarizations are rapidly conducted through gap junctions, which connect the ciliated cells (Gilula and Satir, 1971; Mackie *et al.*, 1974). Additionally, some animals appear to have a finer degree of control of ciliary beating. For example, the ciliary beating frequency of lateral cilia of *Mytilus* gill is variable and dependent upon dopaminergic and serotonergic CNS input (Paparo and Aiello, 1970; Paparo *et al.*, 1975; Aiello *et al.*, 1986). Larvae of many marine invertebrates are also capable of modulating the beat frequency of their locomotory cilia (see review by Chia *et al.*, 1984), yet the mechanisms by which gradual changes in ciliary beat frequency are generated are not well understood.

Veliger larvae use the pre-oral (locomotory) cilia of the velum to move through the water column. Upward swimming is periodically interrupted, either spontaneously or upon contact with "foreign" objects, by sudden ciliary arrests whereupon the veliger rapidly sinks (Garstang, 1929). These ciliary arrests have long been assumed to be under neuronal control because neuronal processes, emerging from the cerebral ganglion, ramify across the velum to the pre-oral ciliated cells (Carter, 1926; Carter,

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1928; Werner, 1955; Fretter, 1967) and form chemical synapses with them (Mackie *et al.* 1976). Electrical activity recorded extracellularly and intracellularly from pre-oral ciliated cells shows no spiking activity during normal ciliary beating, but large depolarizations occur one-for-one with velum-wide ciliary arrests (Mackie *et al.*, 1976). These arrests are absent when the velum is isolated from the rest of the veliger. Pre-oral cell action potentials appear to be Ca^{++}-dependent since elevated Ca^{++} causes longer duration ciliary arrests and addition of Co^{++} blocks arrests (Korobtsov and Sakharov, 1971). Serotonin has a cilio-excitatory effect (Koshtoyants *et al.*, 1961) and an unknown "inhibitory substance," extracted from nudibranch veligers, causes ciliary arrest (Buznikov and Manukhin, 1962).

Some veligers are also capable of finer control of ciliary beating. This control is usually expressed as a gradual decrease in the frequency of the beating cilia. Veligers are thus able to slowly descend with cilia beating at "a reduced level" (Cragg, 1980). Furthermore, there is evidence that veligers are capable of integrating sensory information to alter locomotory and feeding behavior since the beating "vigor" and "rate of [food] collection" by pre-oral cilia varies with the degree of satiation (Fretter and Montgomery, 1968). During this study, we developed a new preparation, exploiting the large, pre-oral ciliated cells of a gastropod veliger larva, to examine the mechanisms by which the larval nervous system modulates ciliary beating and hence controls locomotion.

We demonstrate that excitatory input from the CNS modulates the inherent beating frequency of pre-oral cilia of *Calliostoma ligatum* (Gould, 1849) veligers. We also show that the ciliated cells are electrically coupled to each other, a property which may ensure the velum-wide synchronization of rapid ciliary arrest. These features endow the veliger with two distinct types of locomotory behavior.

Materials and Methods

Larvae were raised in the laboratory. Adult *Calliostoma ligatum,* collected from San Juan Island, Washington, were spawned in separate bowls, containing 20°C, coarse-filtered seawater. Animals were placed foot up and usually spawned between 30 min to 1 hour later. Gelatinous egg strands were dissociated by repeatedly drawing the egg mass into a Pasteur pipette. Eggs were then transferred to additional bowls at a concentration of several hundred eggs per bowl. Approximately 2 ml of sperm from several different males were diluted in 500 ml of filtered seawater. Eggs were fertilized by adding about 0.5 ml of diluted sperm to each bowl. Developing veligers were kept on a seawater table at 10–12°C. Seawater in the bowls was changed twice daily and unfertilized eggs and moribund veligers were removed. Veligers developed rapidly within their egg capsules and usually hatched and began swimming around the bowl after about 5 days. We found that the electrical properties of pre-oral ciliated cells and ciliary activity of veligers as young as 72 hours post-fertilization were nearly identical to that of older veligers, but younger veligers did not retract into their shell as readily. For this reason, we often used these younger veligers for intracellular recordings after excapsulating them with sharpened tungsten needles.

Hatched or excapsulated larvae were held in position and manipulated for recording by attaching a small bore suction (polyethylene tubing) electrode to the shell or foot, or by placing the shell into a small depression in the Sylgard- (Dow Corning) lined recording dish. The frequency of ciliary beating was monitored by holding a larva in the beam of a low power laser (Spectra Physics Model 155; 0.95 mW, 632.8 nm). The beam was aimed at a photodiode and the beating cilia on one velar lobe alternately bisected the beam (Fig. 1D). Voltage changes across the photodiode were

amplified by an AC-coupled preamplifier (Grass Model P-15) with the low pass filter set for 1 s (the longest τ possible). Stroboscopic measurements of changes in ciliary beat frequency were made with a Chadwick-Helmuth Strobex. Conventional intracellular recording techniques were used to record from pre-oral ciliated cells (3 *M* KCl-filled glass electrodes; 20–30 MΩ). For dye injection, electrodes were tip-filled with 5% Lucifer Yellow CH (Sigma) in distilled water and back-filled with 1 *M* $LiCl_2$ (90–100 MΩ). Dye was injected with pulsed hyperpolarizing current (0.5–1.0 nA) for up to ten minutes. Lucifer Yellow-filled velar cells were viewed live with a fluorescence microscope and photographed. All recordings were made in 15–18°C seawater unless otherwise stated. Artificial seawater was used for experiments requiring altered ionic content. Normal artificial seawater was composed of 430 m*M* NaCl, 10 m*M* $CaCl_2$, 10 m*M* KCl, 30 m*M* $MgCl_2$, 20 m*M* $MgSO_4$, 10 m*M* Tris HCl pH = 7.8.

Larvae were processed for electron microscopy by first anesthetizing them in a 1:1 mixture of seawater and isotonic (0.33 *M*) $MgCl_2$ and then fixing in a solution containing 2.0% TEM grade formaldehyde, 1.5% glutaraldehyde, 0.2 *M* sodium phosphate (monobasic), 0.2 *M* sodium phosphate (dibasic) buffer, pH = 7.2 on ice. Larvae were rinsed, post-fixed in 2% osmium tetroxide in the same buffer, dehydrated through graded alcohols and propylene oxide, and embedded in EPON 812. Sections were stained with uranyl acetate and lead citrate.

Results

Ciliary beating and arrest behavior

The pre-oral cilia of *C. ligatum* larvae beat in laeoplectic metachronal waves (Fig. 1A, B) with a frequency of 5–7 Hz (Fig. 1D). This ciliary beating propels larvae upward with the velum and foot leading and the shell trailing. Stoppages of the pre-oral cilia (ciliary arrest) occur either spontaneously, or when larvae contact the air-water interface or other obstacles. Ciliary arrest is characterized by a synchronized stoppage of all pre-oral cilia and varying degrees of contraction of the velar lobes. Cilia are held in a cone-shaped tuft over the disc of the velum (Fig. 1c) during arrest, and thus reside at the beginning of the effective stroke. After a few seconds, the pre-oral cilia begin to beat and the metachronal wave is re-established. Although the arrest of all cilia is synchronized, the resumption of ciliary beating is not always uniform around the velum. Clearly the cilia of some portions of the velum start beating before others. Strong or repetitive stimuli may also cause a withdrawal of the velum and foot into the shell. Recovery from this contracted position usually takes longer than recovery from a simple ciliary arrest.

Ciliary arrests, exhibited by encapsulated or free-swimming larvae, are often rhythmical. A sampling of ten different larvae, all of which were about 96 hours old and still in egg capsules, showed 13.9 to 22.1 ciliary arrests per minute. The interval between arrests is usually very regular. As an example, one larva showing an arrest frequency of 17.6 arrests/min had a mean (±SE, n = 31) period of 3.46 (0.07) seconds. Free-swimming veligers also showed rhythmical arrests, but the rhythmicity was often interrupted by external factors such as debris, air-water interface, or other veligers. In addition to these coordinated velum-wide arrests, we have observed a general slowing of ciliary beating. The slowing in the sinking rate or upward swimming that we have observed in free-swimming veligers appears to be due to this general slowing of ciliary beating.

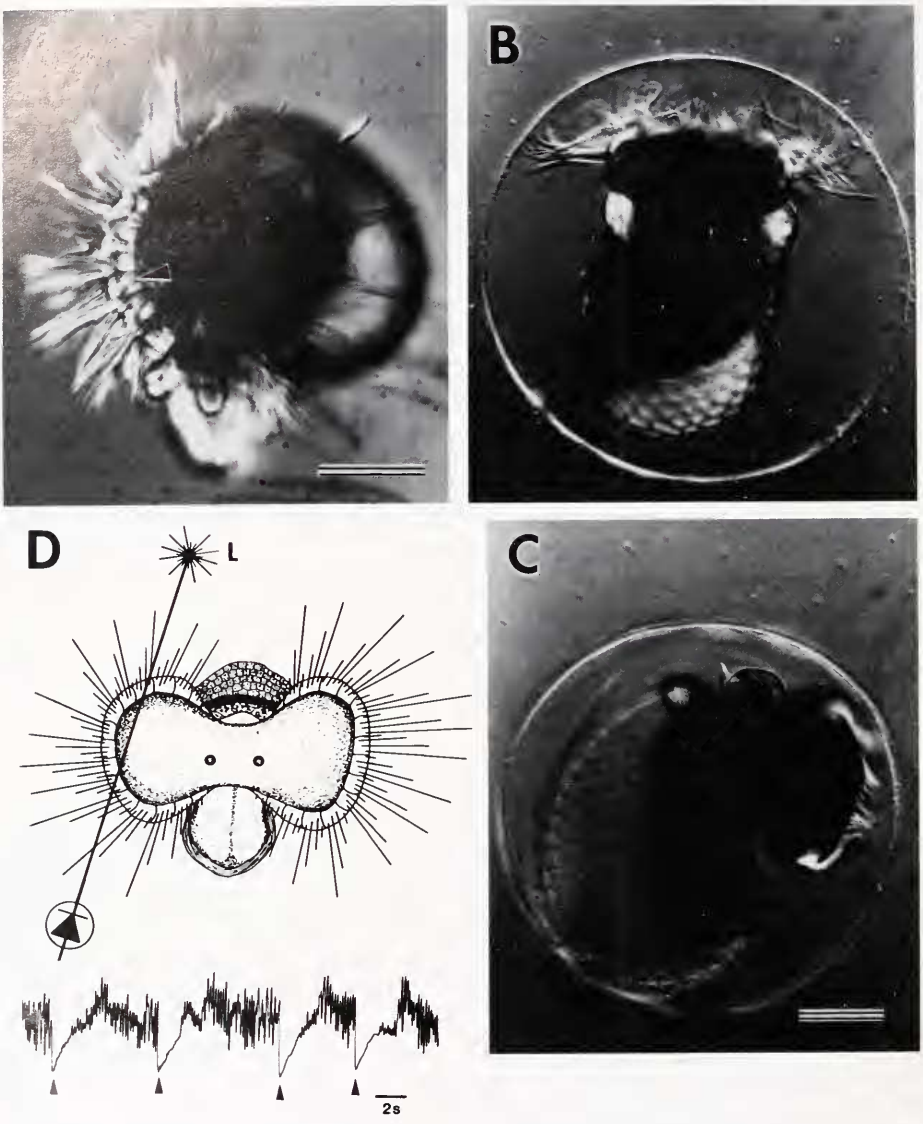

FIGURE 1 A–D. A. Free-swimming veliger of *Calliostoma ligatum* showing the laeoplectic metachronal waves of the pre-oral cilia. Waves move clockwise in this view. Note the outlines of individual pre-oral ciliated cells (arrow). Scale = 100 μm. B. 96-hour-old veliger in egg capsule showing normal ciliary beating. C. Same larva as in B, but with cilia in the "arrested" position. Cilia are held in the characteristic conical tuft. Note that the foot and velum are still extended, but severe stimuli may cause their withdrawal into the shell. Scale for B and C = 100 μm. Light micrographs were made using a Zeiss strobe flash. D. Laser arrangement used to monitor the frequency of metachronal waves and ciliary arrest. Laser beam is aimed at a photodiode and the veliger is positioned so that the cilia alternately bisect the beam. The large downward deflections in the voltage record (arrows) correspond to velum-wide ciliary arrests. The small oscillations between arrests represent the metachronal waves. L—laser.

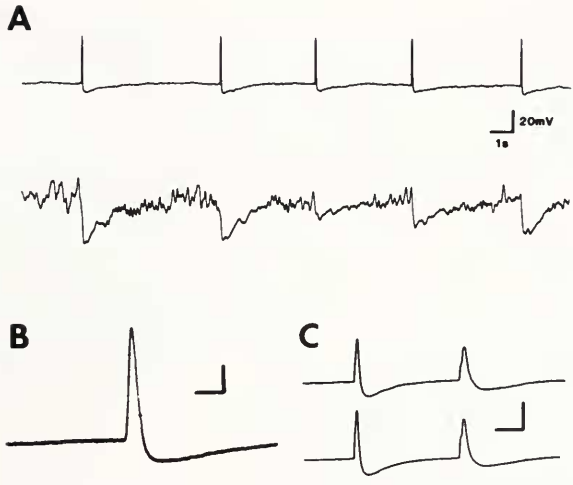

FIGURE 2 A–C. Intracellular electrode recordings from pre-oral ciliated cells of *Calliostoma ligatum* veligers. A. Single electrode recording from a ciliated cell (top) with a simultaneous photodiode voltage record (bottom) of ciliary beating and arrests. Note that each spike corresponds 1:1 to a large downward deflection of the diode voltage record, indicating a velum-wide ciliary arrest. The third spike also caused a velum-wide arrest, but the veliger had moved slightly to a position less than optimal for detecting ciliary arrest. Cilia gradually begin to beat within 1–2 s after a spike. Recommencement of ciliary beating is shown by the small oscillations which gradually increase in amplitude until the normal metachronal wave is re-established. B. Single oscilloscope sweep of a spontaneous, ciliated cell action potential. Note the absence of EPSP activity on the depolarizing phase. Horizontal scale = 50 ms, vertical scale = 10 mV. C. Dual electrode recording from opposite velar lobes, showing synchrony of action potentials. Note the distinct shoulder on the rising phase of the smaller amplitude second spike. Horizontal scale = 200 ms, vertical scale = 20 mV.

Physiology of pre-oral ciliated cells

Intracellular recordings from pre-oral ciliated cells show a resting potential of −60 mV. These cells exhibit no spiking activity or membrane potential oscillations during normal ciliary beating. However, coordinated, velum-wide arrests of pre-oral cilia correlate 1:1 with action potentials (Fig. 2A). These spikes have a positive phase lasting 40 ms, a peak amplitude of 45–50 mV, and a 10 mV hyperpolarizing undershoot lasting 700 ms (Fig. 2B). The hyperpolarizing phase is usually absent upon initial penetration of a cell, although spikes, lacking this phase, still cause ciliary arrest. Recordings from some ciliated cells show summing excitatory postsynaptic potentials (EPSPs) leading to an action potential, while others show no such EPSPs (Fig. 2B, C). The absence of EPSPs in some spikes may indicate that the synaptic site(s) is far enough away from the recording site such that EPSPs have decayed. We do not know if every pre-oral cell is innervated, but these results would suggest that they are not. Spontaneous spikes recorded from opposite velar lobes are well synchronized (Fig. 2C), showing about a 1 ms peak-to-peak delay. A stimulated spike propagates at about 20 cm/s. About 1–2 s after a spike, cilia slowly begin to beat again and the metachronal wave is re-established. However, at spiking frequencies greater than approximately 0.4 Hz, cilia remain arrested or twitching in the arrested position. Spike bursts rarely occurred at higher frequencies, but Figure 2C shows one such example. Note that the amplitude of the second spike in the burst is slightly reduced. The absolute refractory period for stimulated spikes is about 200 ms.

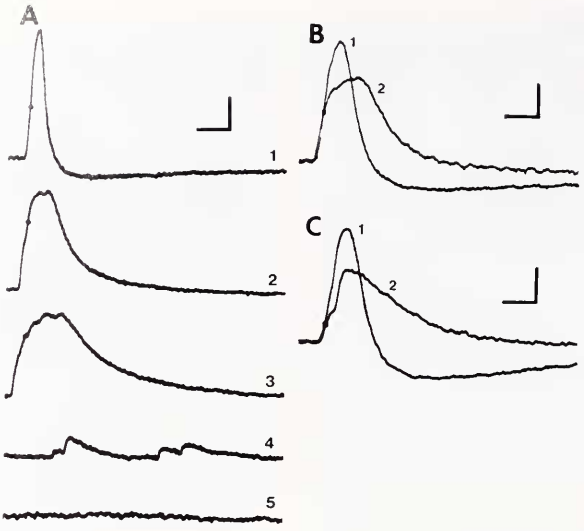

FIGURE 3 A. Intracellular electrode recording from pre-oral ciliated cells of *Calliostoma ligatum* veligers. Horizontal scale = 50 ms. (1, 2, 3) 200 ms (4, 5), vertical scale = 10 mV. 1. Single oscilloscope sweep of a spontaneous action potential in normal seawater. Note the short duration positive phase and long duration hyperpolarizing undershoot. 2. One minute after beginning replacement of normal $CaCl_2$ seawater with 10 m*M* $CoCl_2$ seawater. Note the loss of the hyperpolarized undershoot and the broadening of the spontaneous action potential. Spike still caused a velum wide ciliary arrest. 3. After two minutes, depolarization no longer caused velum-wide ciliary arrest, although localized "stutters" in ciliary beating were visible. 4. Three minutes in 10 m*M* $CoCl_2$ seawater, small spontaneous EPSPs are still present. 5. Five minutes in 10 m*M* $CoCl_2$ seawater. All synaptic activity is abolished. Spikes could not be initiated by intracellular stimulation. Upon return to normal seawater, spikes return as in 1. Same electrode penetration for 1–5. B. Spontaneous action potential of pre-oral cells in normal seawater (1) and about 5 minutes after the addition of 5 m*M* 4-aminopyridine (2). Horizontal scale = 20 ms, vertical scale = 10 mV. C. Action potential of pre-oral cells in normal seawater (1) and two minutes after the addition of 10 m*M* tetraethylammonium chloride. Horizontal scale = 20 ms, vertical scale = 10 mV.

Pre-oral ciliated cell action potentials are reversibly reduced in amplitude and are eventually abolished when $CaCl_2$ is replaced by an equimolar concentration of $CoCl_2$ (Fig. 3A). Cilia beat continuously and action potentials could not be elicited by intracellular electrode stimulation in 10 m*M* $CoCl_2$ seawater. Increasing concentrations of Mg^{++} produced a similar effect, but even at 120 m*M* Mg^{++}, a 5–10 mV depolarization was still detectable. It appears that Ca^{++} alone is responsible for the depolarization since a reduction of Na^+ to 25% of normal seawater (107.5 m*M* NaCl and 322.5 m*M* choline chloride) had no effect on spike amplitude or duration (not shown). The initial effect of the addition of Co^{++} on the spike is a broadening of the positive phase and a loss of the distinct hyperpolarizing undershoot (Fig. 3A). These effects suggest that Ca^{++}-dependent potassium currents are responsible for the hyperpolarization phase and these currents might be blocked by K^+ channel blockers (Hille, 1984). Addition of 5 m*M* 4-aminopyridine (4-AP) or 10 m*M* tetraethylammionium chloride (TEA) (Sigma) mimics the initial Co^{++} effects by eliminating the hyperpolarizing undershoot and broadening the spike (Fig. 3B, C).

Pre-oral ciliated cells are electrically coupled to each other. Depolarizing and hyperpolarizing current injected into one cell causes a proportional depolarization and hyperpolarization in neighboring cells (Fig. 4A). A brief pulse of suprathreshold positive current can produce a spike that propagates throughout the velum and causes

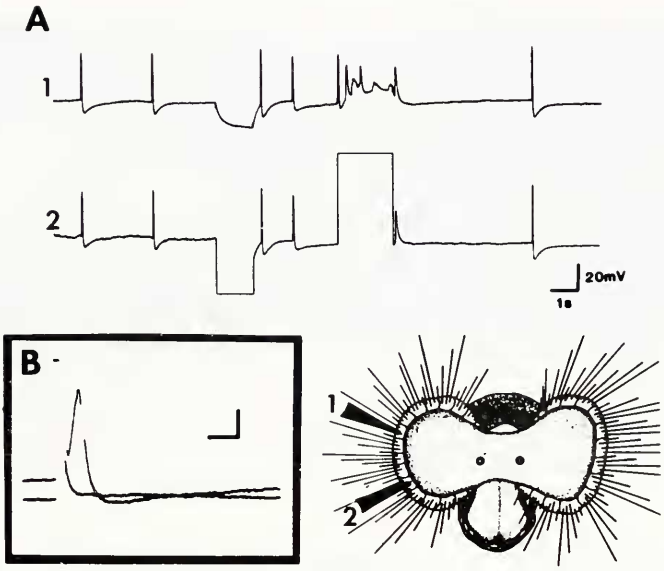

FIGURE 4 A, B. Intracellular electrode recordings from pre-oral ciliated cells of *Calliostoma ligatum* veligers. A. Simultaneous dual electrode recording demonstrating electrical coupling between ciliated cells. Hyper- and depolarizing current (±5 nA) injected into electrode #2 causes a corresponding hyper- and depolarization of a neighboring ciliated cell (electrode #1). Similar results were observed when electrodes were located on opposite velar lobes. Note the synchrony and similarity of the spontaneous spikes in both recordings. Electrode bridge was not balanced. B. Successive sweeps of stimulated ciliated cell action potentials, using the same electrode penetration. In the first sweep, a brief subthreshold pulse of depolarizing current caused no spike. A spike was elicited in the second sweep when a suprathreshold current pulse was injected into the cell. Horizontal scale = 50 ms, vertical scale = 10 mV.

ciliary arrest (Fig. 4B). Ciliated cells are dye coupled to each other as Lucifer Yellow injected into one cell rapidly spreads throughout all pre-oral ciliated cells (Fig. 5).

We found that it was possible to modulate the inherent frequency of ciliary beating by injecting ramp depolarizing current into a ciliated cell. As the membrane potential of a ciliated cell is depolarized from resting potential, cilia of the impaled cell and those immediately adjacent to it begin to slow. As the cell is depolarized further, a wave front of slowing cilia spreads symmetrically about the electrode until all pre-oral cilia slow and eventually stop beating (Fig. 6a, c). If the stimulus current is turned off rapidly, cilia of all cells begin to beat again. However, if the current is slowly reduced back to zero, then the cilia far from the electrode begin to beat first, followed by those close to the electrode. From these results, it appeared that the frequency of pre-oral ciliary beating was dependent upon the level of depolarization of the ciliated cell membrane, although the laser arrangement shown in Figure 1D did not have the resolution to detect small changes in ciliary beat frequency that accompanied the slow depolarizations. We tried to document this phenomenon by making stroboscopic measurements of changes in ciliary beat frequency while depolarizing the pre-oral cells. This was done by increasing the concentration of KCl in the bath to depolarize the velum as a whole, rather than from a point source as in Figure 6A, C. Increases in the concentration of KCl to 0.5, 0.75, 1.0, 1.25, 1.75, 2.0 m*M*, above normal seawater linearly depolarizes the resting potential by 4, 6, 7, 8, 10, 11 mV, respectively. While depolarizing the membrane potential, however, the spiking frequency

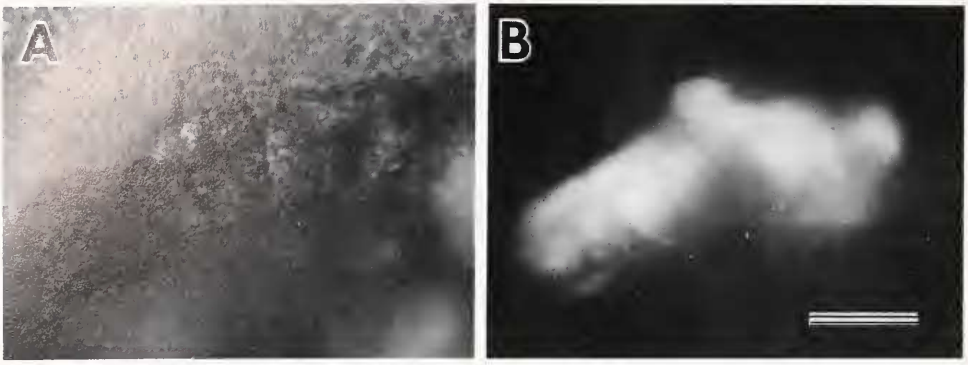

FIGURE 5 A, B. Lucifer Yellow dye coupling of pre-oral ciliated cells of live *Calliostoma ligatum* veligers. A. Brightfield micrograph of the velum. Cilia extend toward the top of the photo. B. Fluorescence micrograph of the same portion of the velum as A. A single cell was filled and dye spread throughout all cells. Note the outlines of individual cells. Scale = 30 μm.

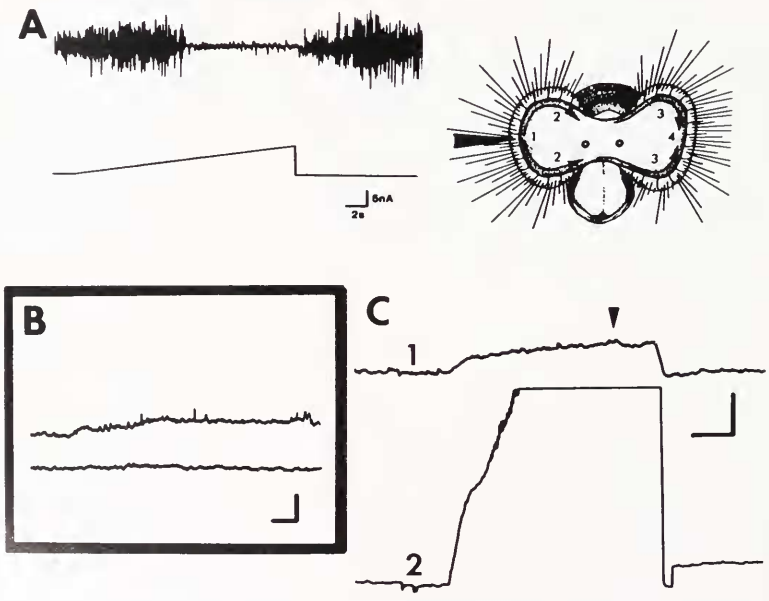

FIGURE 6 A–C. Intracellular electrode recordings from pre-oral ciliated cells of *Calliostoma ligatum* veligers. A. Ramp depolarizing current injected into a single ciliated cell causes an initial slowing of cilia at position 1. As the ramp continues, the wave front of slowing cilia follows the arrows and cilia at position 2 begin to slow when those around 1 have stopped. This wave front passes through 3 to 4, whereupon all cilia have stopped. Laser record (top) shows that when the current is terminated, cilia begin to beat again. The laser beam passed through position 3 and thus detected the arrest of cilia in this area alone. All cilia were arrested before the current ramp reached its maximum. B. Simultaneous dual electrode recording from ciliated cells on opposite velar lobes. Top trace shows a spontaneous slow depolarization with numerous high frequency EPSPs while bottom trace remains at resting potential. Horizontal scale = 1 s, vertical scale = 10 mV. C. Simultaneous dual electrode recording from ciliated cells on opposite velar lobes. Depolarizing current (up to 5 nA) was gradually injected into electrode #2. Electrode #1 shows the corresponding depolarization. Cilia began to slow during the depolarization and eventually arrested at the position of electrode #1 at the arrow. Electrode bridge was not balanced. Horizontal scale = 2 s, vertical scale = 20 mV.

increases and consequently the cilia remain arrested, or the metachronal wave was disrupted to the extent that visual synchronization was impossible. This fact hampered the visual measurement of ciliary beat frequency for a range of membrane potentials, and thus we were only able to document beat frequency reductions in response to small depolarizations. In one veliger, ciliary beat frequency in normal seawater was 7.2 Hz. This frequency was reduced by 13% to 6.3 Hz after increasing the concentration of KCl by 1.5 m*M*. This increase in KCl depolarized the −60 mV resting potential by 9 mV or 15%.

We have observed occasional spontaneous, high frequency EPSPs that are superimposed on long duration depolarizations (Fig. 6B). These slow depolarizations likely correspond to the general slowing of ciliary beating as described above, although we could not monitor ciliary beat frequency during dual intracellular electrode recordings. Slow depolarizations may be recorded on one velar lobe and not the other (Fig. 6B), whereas spikes are always observed velum-wide. These collective findings suggest, although admittedly do not prove unequivocally, that the frequency of pre-oral ciliary beating is dependent upon the depolarization level of the ciliated cell membrane and this level is modulated by excitatory synaptic input.

Morphology of pre-oral ciliated cells

The morphology of pre-oral ciliated cells of *C. ligatum* are very similar to those of *Mangelia spp.*, which have been well described by Mackie *et al.* (1976). Thus, only a brief description of these velar cells of *C. ligatum* will be given here. Pre-oral ciliated cells are characterized by the presence of numerous scythe-like compound cilia (Fig. 7A). There are usually about 25 of these cilia per cell and each one is composed of approximately 50 individual cilia. Each individual cilium is anchored in the cell by a distinct basal body and a long striated rootlet (Fig. 7B). The rootlets are robust and extend well into the cell. Numerous mitochondria are concentrated just below the basal bodies. Another distinct feature of these cells is the presence of numerous, large lipid vacuoles, concentrated near the base of the cell (Fig. 7A, D). These vacuoles appear to be similar to the "reserve food vacuoles" described by Carter (1928). Distinctively less dense sheath cells lie on the oral and aboral sides of ciliated cells as in *Mangelia spp.* (Mackie *et al.*, 1976). Sheath cells bear numerous microvilli and only a few simple cilia. Lipid vacuoles are conspicuously absent from sheath cells. Pre-oral ciliated cells are connected to each other by numerous gap junctions and a few septate demosomes (Fig. 7B, C). Ciliated cells are richly innervated by axons containing electron-lucent vesicles (Fig. 7D). Most of these neuro-ciliary synapses are found at the proximal end of the cells, although we have observed some distally.

Discussion

We have provided evidence to suggest that the beating frequency of the locomotory, pre-oral cilia of competent veligers of *C. ligatum* is dependent upon the level of depolarization of the ciliated cells' membrane and that this level is modulated by excitatory neuronal input. Pre-oral cilia appear to have an inherent beating frequency which is seen at resting potential, or when the ciliated cells are isolated from excitatory inputs either physically by dissociation or chemically in high Mg^{++} solutions. The inherent beating frequency is reduced by excitatory inputs, presumably originating from the cerebral ganglion. This ganglion is highly developed in early veliger stages of *Aplysia californica* (Kandel *et al.*, 1980) and its axons have been shown to innervate the velar ciliated cells in several veligers (Carter, 1926, 1928; Mackie *et al.*, 1976).

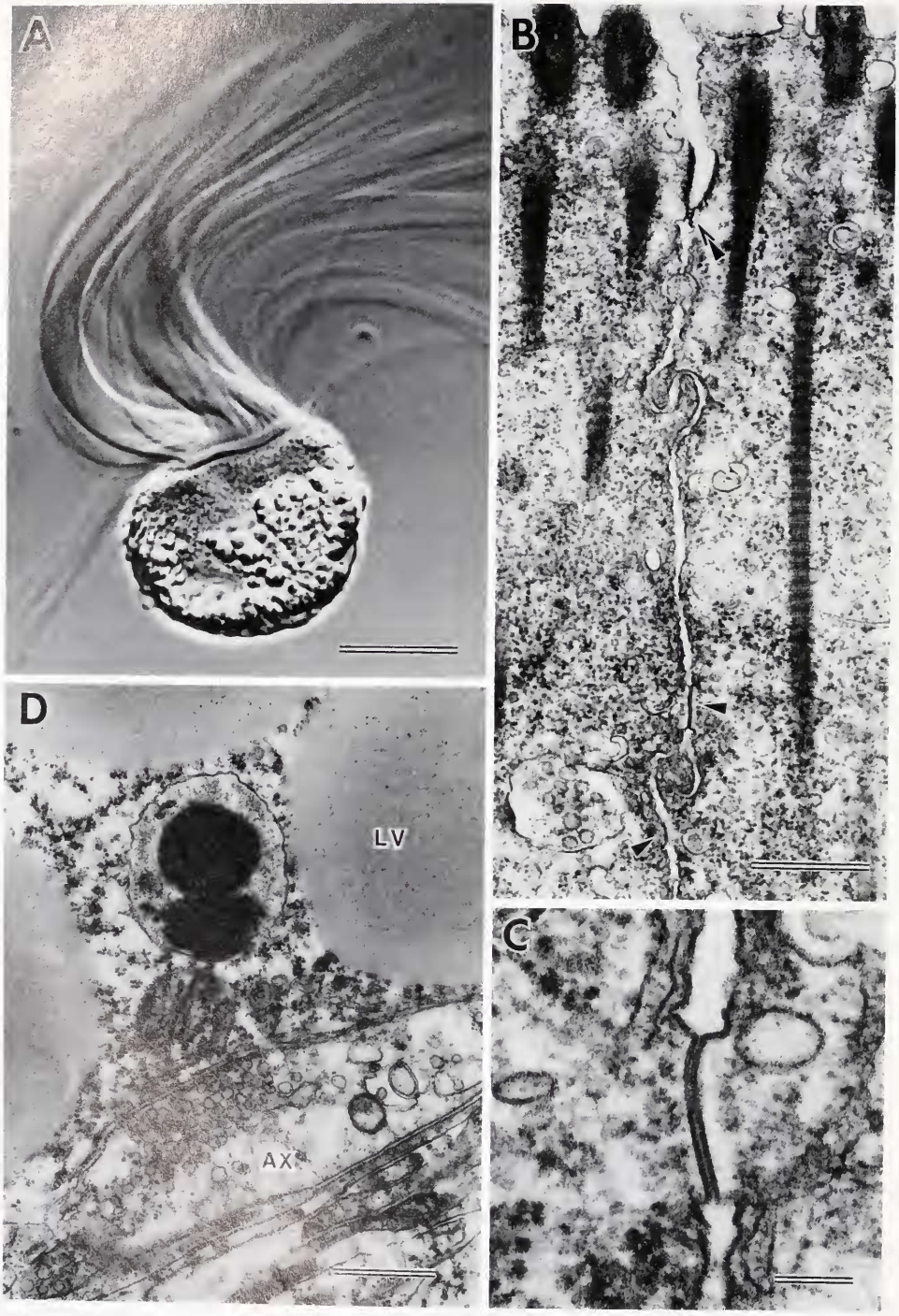

FIGURE 7 A–D. A. Side view of a single, isolated pre-oral ciliated cell from *Calliostoma ligatum*. Aboral end is to the right, oral end is to the left. Effective stroke is from left to right. Note the scythe-like compound cilia which lie in rows orthogonal to the direction of beat. Prominent spheres at the base of the

We also find many neuro-ciliary synapses (Fig. 7D), but we have not determined the origin of these axons, nor do we know if all pre-oral cells are innervated.

Control of locomotion

Veligers are capable of modulating the inherent frequency of ciliary beating to yield two distinct types of locomotory behavior. In the first type, normal ciliary beating is periodically interrupted by rapid, coordinated, velum-wide ciliary arrest (Figs. 1D, 2A). Coordinated velum-wide ciliary arrests are caused by a ciliated cell action potential, which results from summing EPSPs. This spike propagates rapidly throughout the electrically coupled pre-oral cells (Fig. 2C). Cilia remain arrested with spiking frequencies of 0.4 Hz or greater. One to two seconds after the spike, cilia begin to beat and during normal ciliary beating, the membrane potential remains at resting potential and little synaptic activity is observed. This modulation of ciliary beating is similar to that in other veligers (Mackie *et al.*, 1976) and other metazoan ciliated systems (Mackie *et al.*, 1974; Saimi *et al.*, 1983a; Arkett, 1987) where a rapid stoppage of ciliary beating is advantageous (see below).

We have provided several pieces of evidence to suggest that the action potential of the pre-oral ciliated cells is dependent upon an influx of Ca^{++}. Replacement of Ca^{++} by Co^{++} reduces the amplitude and eventually blocks the spike and prevents ciliary arrest. (Fig. 3A). This finding corroborates the study by Korobtsov and Sakharov (1971) which showed that Co^{++} blocks ciliary arrest behavior. We have also shown that the K^+ channel blockers, 4-AP and TEA, eliminate the long hyperpolarizing undershoot and broaden the spike, suggesting Ca^{++}-dependent K^+ channels are present in the pre-oral cells. After the large influx of Ca^{++} during the depolarizing phase of the spike, these K^+ channels may be activated, thereby hyperpolarizing the cell and preventing further Ca^{++} entry (Hille, 1984). As the internal Ca^{++} concentration is reduced, cilia may resume beating and a second spike may be initiated. These large ciliated cells should prove to be a valuable preparation for more fully characterizing the currents involved in ciliary arrest and the resumption of beating.

The second type of locomotion is produced by a gradual slowing of ciliary beating, rather than by full velum-wide ciliary arrests. High frequency EPSPs generate long duration, slow depolarizations of the ciliated cells (Fig. 6B, C). As the ciliated cells gradually depolarize, the frequency of ciliary beating appears to drop. We have not observed veligers of this species to use this method of modulating ciliary beating frequency to stop ciliary beating, only to slow it. Localized excitatory input to pre-oral cells can alter the ciliary beating frequency of one velar lobe and not the other (Fig. 6B). This differential ciliary beating may be used by the veliger in conjunction with velar muscle contractions to make turns in the swimming path.

We have demonstrated that an action potential is actively propagated throughout the ciliated pre-oral velar epithelium. The rapid depolarizations associated with ciliary arrests in ascidian branchial basket (Mackie *et al.*, 1974; Arkett, 1987) and *Mytilus* gill (Murakami and Machemer, 1982; Saimi *et al.*, 1983a) appear to be conducted

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cell are lipid vacuoles. Scale = 25 μm. B. Electron micrograph of the distal portion of two ciliated cells connected by gap junctions (single arrows) and a septate desmosome (double arrow). Note the robust striated rootlets. Scale = 0.5 μm. C. A single gap junction connecting two pre-oral ciliated cells. Scale = 0.1 μm. D. An axon (AX) containing electron lucent vesicles at the base of a ciliated cell. LV—lipid vacuole; Scale = 0.5 μm.

passively through gap junctions connecting ciliated cells. We have also provided evidence that suggests a dependence of ciliary beat frequency on the level of depolarization of the ciliated velar cells, although unequivocal demonstration of this point was not possible using the intact veliger. The mechanisms for *Mytilus* gill appear to differ since Saimi *et al.* (1983b) stated that the ciliary beat frequency is independent of the ciliated cell membrane potential. Yet, Aiello *et al.* (1986) showed that dopamine caused a dose-dependent decrease in the endogenous ciliary beating frequency of *Mytilus* lateral cilia, but they did not monitor ciliated cell membrane potential. In *Paramecium,* forward swimming increases as the membrane potential becomes more hyperpolarized (Machemer, 1974; Bonini *et al.,* 1986). We found that hyperpolarization of veliger pre-oral ciliated cells has no apparent effect on beating frequency. Pre-oral cilia never appear to beat faster than their inherent frequency.

Importance of electrically coupled ciliated cells

The pre-oral ciliated cells are electrically coupled to each other (Figs. 4A, 5) by numerous gap junctions between the cells (Fig. 7B, C). Strong electrical coupling should ensure that EPSPs will summate spatially from velum-wide excitatory input to ciliated cells and temporally due to a reduction in the junctional shunting of current between the cells and a longer time constant (Getting, 1974; Berry and Pentreath, 1977). These features lead to a regenerative action potential in the pre-oral cells. Electrical coupling of these cells should ensure that an action potential generated at one point on the velum will propagate rapidly throughout the pre-oral cells, thereby synchronizing the arrest of all pre-oral cilia (Figs. 2C, 4A, B). This property of rapid junctional transmission is one of the most important features of electrically coupled systems (Bennett, 1977). However, we cannot rule out an alternative explanation, namely that ciliary arrest in the two velar lobes is synchronized by bilaterally symmetric pairs of neurons in the cerebral ganglion.

The electrical coupling properties of pre-oral cells may also make possible small, local changes in the membrane potential without spikes. Local CNS input may depolarize a portion of the velum, but because the input resistance of the velar cells is low, current is shunted throughout the pre-oral cells and no spikes are generated. However, such local input can result in long duration, slow depolarizations (Fig. 6B, C) and thus a differential slowing of ciliary beat frequency on different sides of the velum.

Functional significance of locomotory control

We have demonstrated that the veligers of *C. ligatum* are capable of controlling locomotory behavior. This control appears to be expressed in two different types of locomotion. The alternating upward swimming and sinking upon ciliary arrest is probably the best known type of locomotion and it has been proposed as a mechanism for regulating position in the water column (Richter, 1973; Cragg and Gruffydd, 1975; Hidu and Haskin, 1978). Net upward movement could be accomplished by decreasing the frequency or duration of ciliary arrests, consequently increasing the amount of time spent swimming. Since veligers generally sink faster than their maximum ascent speed (Cragg, 1980; Chia *et al.,* 1984), an increase in the frequency of ciliary arrest should result in a net downward movement. Although the main use of this type of locomotion may be in regulating diel vertical migration patterns (Richter, 1973), it may also be important as a defence mechanism. Contact with "foreign" objects or potential predators might cause a rapid ciliary arrest and sometimes a retraction of the body into the shell, whereupon the larva sinks rapidly (Fretter, 1967).

The second type of locomotion is characterized by more subtle changes in normal ciliary beating. The gradual, velum-wide slowing of locomotory cilia without full arrest would enable the veliger to either swim slowly upward or sink at a slower rate than during full ciliary arrest (Richter, 1973; Cragg and Gruffydd, 1975). This behavior appears to be closely linked to the veliger's satiation level (Fretter and Montgomery, 1968) and may enable feeding veligers to remain within a zone of abundant food. The differential ciliary beating of velar lobes observed in *C. ligatum* in conjunction with muscle contraction of the velum may enable the veliger to vary its swimming direction.

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